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Research Article

Exploration The Candidates of Xenobiotic Degrading Indigenous Bacteria from Probolinggo City Landfill by Using Next Generation Sequencing (NGS)

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Abstract

Soil bacteria from tropical environments play a significant role in resolving various environmental issues, including biodegradation. Exploratory research on biodiversity is crucial to develop and harness the potential of different types of soil bacteria that are highly abundant. The bacterial diversity in landfills is typically high due to the decomposition of organic and inorganic waste, creating a favorable medium for the growth and development of soil bacteria. This study aims to assess the candidates of xenobiotic degrading indigenous bacteria from the Probolinggo City landfill using Next Generation Sequencing (NGS) method. The research stages include: 1) sampling, 2) isolation of genomic DNA from samples using the ZymoBIOMICS DNA MiniPrep Kit from Zymo Research, 3) amplification of isolated DNA with primers 16S 27F – 1429R, 4) sequencing the results of DNA amplification with NGS, 5) downstream analysis of the results using software Pavian Krona Tools, and 6) narrative analysis review to identify the candidates of xenobiotic degrading indigenous bacteria. The results show that soil samples from the Probolinggo City landfill exhibited a high diversity of bacterial communities. Based on NGS analysis, 2400 bacterial species were identified, comprising 56 genera, 17 orders, 4 classes, and 4 phyla, with respective abundances of *Proteobacteria* (70%), *Firmicutes* (15%), *Planctomycetes* (2%), and *Cyanobacteria* (0,3%). Based on the narrative analysis review, several bacteria in the Probolinggo City landfill exhibited potential as: 1) polypropylene-degrading bacteria, including *Bacillus cereus, B. licheniformis and B. thuringiensis.* 2) styrofoam degrading bacteria, namely *Bacillus amyloliquefaciens, Bacillus cereus, Bacillus firm*us and *Pseudomonas aeruginosa*. 3) total ammonia nitrogen (TAN) reducing bacteria, including *Bacillus megaterium*. 4) pesticide degrading bacteria *Profenofos* and *Chlorantraniliprole*, including *Bacillus stearothermophilus*. and 5) tannic acid degrading bacteria, including *Pantoea dispersa*. These results indicate that the Probolinggo City landfill is a good habitat for various xenobiotic-degrading bacteria, then the isolation of specific bacteria can be designed using an appropriate selective medium.

Keywords: NGS, indigenous bacteria, xenobiotic degrading, landfill.

1. INTRODUCTION

The global environment's poisoning by a complex mixture of xenobiotics has become a major environmental threat worldwide [1]. Xenobiotic contaminants such as azo dyes, phenolics, polycyclic aromatic hydrocarbons (PAHs), halogenated compounds, personal care products (PCPs), pharmaceutical active compounds (PhACs), pesticides, nitroaromatic

compounds, triazines, and chlorinated compounds have a detrimental impact on the environment due to their long-lasting impacts [2]. In addition, these pollutants have teratogenic, carcinogenic, mutagenic, and toxic effects on all organisms. Therefore, removing toxic undegradable xenobiotics from the environment is necessary. The degradation of xenobiotics by microbes is an evolutionary trait by which microbes survive in the presence of toxins utilizing them as carbon, phosphorus, sulfur, or nitrogen source [3,4]. The ability of microorganisms to detoxify xenobiotic compounds allows them to thrive in a toxic environment using carbon, phosphorus, sulfur, and nitrogen from the available sources. Biotransformation is the most effective and useful metabolic process to degrade xenobiotic compounds [5].

Bacteria dominate the microbial biomass in the soil, constituting almost half of it [6]. Soil bacteria play a crucial role in environmental bioremediation by biologically breaking down waste into simpler compounds that can be utilized in metabolic processes [7]. This potential is closely linked to the various abilities of bacteria such as amylolytic, lipolytic, antibiosis, proteolytic, cellulolytic, etc. [8]. Soil bacteria are one of the indigenous bacteria, naturally living microorganisms that have various benefits for human beings [9]. Final disposal sites are a promising source for finding soil bacteria due to the presence of abundant organic and inorganic waste that has been buried for an extended period [10]. Previous studies have identified bacterial genera capable of degrading synthetic polymers from landfills, such as *Acetobacter, Paracoccus, Bacillus, Agrobacterium, Zooglea, Lactobacillus, Alcaligenes, Acinetobacter, Microbacterium*, and *Carnobacterium* [11]. Another study from India found five potential isolates of polypropylene-degrading bacteria from a landfill in Okhla, New Delhi, namely *Bacillus cereus, Bacillus licheniformis,* and *Bacillus thuringiensis,* out of 16 isolated bacteria [12]. Indonesia with its rich biodiversity and supportive environment for microbial growth, especially soil bacteria, presents a promising prospect for exploring potential bacterial diversity such as Probolinggo City Landfill**.** It contains three landfill sites or cells, one passive cell, and two active cells, covering a total land area of 17 hectares with a waste volume of 37.5 tons/day.

Commonly used methods for determining diversity of microbial communities are metagenomic analysis molecular techniques and cultured techniques. However, approximately 99% of microorganisms present in the environment cannot be cultured under laboratory conditions because some grow so slowly that it takes months or even years [13]. Limitations of microbial culture can also be affected by dependence on other organisms in nature [14]. Therefore, to analyze the diversity of bacterial communities that cannot be cultured, one of them is through metagenomic analysis. Metagenomics is the direct genetic analysis of the genome in a sample taken from the environment [13]. The principle of metagenomic analysis is based on DNA taken directly from a community in a small ecosystem, then analyzed and identified using phylogenetic marker genes such as the 16S rRNA gene. This gene aims to determine the number of microbes globally [15]. Metagenomic analysis in this study was carried out using informatics-based Next Generation Sequencing (NGS) technology so that it can provide information that is considered adequate and efficient, such as searching for annotations (naming), genome mapping, and further sequence analysis [16]. However, research on soil bacterial biodiversity using the NGS technique has never been carried out in Indonesia. NGS is a sequencing method that produces more significant sequence data in a relatively short time, so this technology is known as high throughput sequencing platform [17]. The choice of primer becomes one of the essential things that affects the results of clear phylogeny in the analysis process [18], so that as a standard, according to Vinje et al. (2015), the full-length 16S rRNA gene (approximately 1500 bp) can be used as a primer for accurate taxonomic identification [18]. Several studies on the application of NGS technology in studying microbial diversity include research conducted by Jarvis et al. (2013) using NGS technology and hypervariable V6 from 16s rDNA, obtained as many as 16,400 Operation Taxonomy Units (OT), including Proteobacteria, Actinobacteria, Bacteroidetes, Cyanobacteria, and Verrucomicrobia [19] Akinsaya et al. (2015) research related to the metagenomic study of endophytic bacteria in aloe vera analysis revealed that Proteobacteria, Firmicutes, Actinobacteria, and Bacteriodetes were the dominant genera [20]. Research with Illumina-MiSeq sequencing of 16s rRNA and 18s rRNA gene amplicons by Wang et al. (2017) found that beneficial microbes including Bacillus, Agromyces, Micromonospora, Pseudonocardia, Acremonium, Lysobacter, Agromyces, Micromonospora, Pseudonocardia, Acremonium, Lysobacter, Mesorhizobium, Microvirga, Bradyrhizobium, Acremonium, and Cheatomium were more abundant in healthy soil than soil infected with wilted bacteria [21]. We can synthesis the exploration to obtain bacterial candidates that have certain potential based on the

data from NGS results and comparative information from Publish or Perish (PoP) 7. This study aimed to find out information on the diversity of soil bacterial communities at the Bestari landfill, Probolinggo, East Java using the Next Generation Sequencing (NGS) technique and is expected to be able to identify the candidates of xenobiotic degrading indigenous that can be developed for use in waste management strategies for environmental bioremediation.

2. RESEARCH METHODS

This study employs a descriptive exploratory method to assess the biodiversity of the bacterial community in the passive zone of the Bestari Probolinggo City landfill, which consists of soil sampling and next generation sequencing (NGS) analysis. NGS analysis begins with isolation of genomic DNA from samples using the ZymoBIOMICS DNA MiniPrep Kit from Zymo Research, then the amplification of isolated DNA with primers 16S 27F – 1429R, sequencing the results of DNA amplification with NGS downstream analysis of the results using software Pavian Krona Tools with library preparation was conducted using Kits from Oxford Nanopore Technology and narrative analysis review to identify the candidates of xenobiotic degrading indigenous bacteria.

Soil Sampling

Sampling was conducted in September 2021 at the Bestari Probolinggo City landfill specifically in the passive cell area that has not been used for waste disposal since 2017. The sampling method employed was purposive sampling, and five collection points were selected. At each point, soil samples were collected using a shovel at a depth of approximately 7-10 cm. Approximately 250 grams of soil were collected at each point to ensure an adequate sample size. The samples from the five collection points were thoroughly mixed and sieved to obtain a homogenous soil sample, resulting in a total of 1 kilogram of soil. The soil samples were then placed in dark bottles and stored in an icebox containing ice gel to maintain a consistent temperature during transportation. Subsequently, the samples were sent to PT. Genetics Science Tangerang for microbial biodiversity analysis using the Next Generation Sequencing (NGS) method.

Next Generation Sequencing (NGS) Analysis

In this study, soil samples obtained from the passive zone of the Bestari landfill, Probolinggo City, underwent DNA extraction and NGS by Genetica Science. Genomic DNA from each sample was isolated using the ZymoBIOMICS DNA Miniprep Kit from Zymo Research. The concentration of DNA was determined using a NanoDrop spectrophotometer and a Qubit fluorometer, required was about 50 ng/µL. Subsequently, the DNA was amplified using a full-length 16S rRNA Polymerase Chain Reaction (PCR) gene targeting the V1-V9 region, with the primers 27F (AGAGTTTGATCCTGGC TCAG)–1429R (GGTTACCTTGTTACGACTT). The amplified DNA was then subjected to sequencing using the GRIDIRON instrument and MinKNOW software version 20.06.9. Base calling of the genetic sequence data was performed using guppy software to generate readable data. Quality control of the data was conducted using a nano plot, and the microbial classification was performed using a classification machine. The downstream analysis involved using the pavian krona tools software to visualize the microbial community data through krona visualization and Sankey diagrams.

3. RESULTS AND DISCUSSION

DNA Extraction and Amplification of 16 full length sRNA Soil Samples from Probolinggo City Landfill

Direct DNA extraction from soil samples using the ZymoBIOMICS DNA MiniPrep Kit requires a minimum sample amount of 250 mg. All cells in the soil sample are lysed using a homogenization method using beads in a lysis buffer. Several purification stages to remove impurities, especially protein, to obtain pure DNA extract. Amplification of the 16S rRNA region contained in the DNA extract using Polymerase Chain Reaction (PCR) with primers forward 27F (AGAGTTTGATCCTGGCTCAG) and reverse 1429R (GGTTACCTTGTTACGACTT), the size of the amplified DNA was confirmed using electrophoresis (Figure 1), showing that several bands appeared. The band parallel to the DNA marker at 1500 bp is a full-length bacterial 16S rDNA fragment, while bands in other areas are predicted to be other DNA fragments recognized by the two primers but not 16S rDNA. All amplicons obtained were sequenced using next-generation sequencing (NGS) techniques. NGS works by combining sequencing in parallel mode, enabling the reading of billions of DNA or RNA fragments simultaneously, significantly speeding up the sequencing process. DNA amplification results were sequenced with the GridION instrument using MinKNOW software version 20.06.9. The base calling was done using Guppy

software to visualize the data from genetic sequence data resulting in ±2500 sequences. Next, this collection of sequence data goes through a quality control stage using a nano plot to ensure that only bacterial sequences enter the analysis stage using Pavian Krona tools software to visualize bacterial community data using Krona visualization & Sankey diagrams.

Bacterial Biodiversity Result of Next Generation Sequencing (NGS) Analysis

The NGS analysis of the passive cell soil samples from the Bestari landfill revealed the presence of four phyla, namely Proteobacteria (70%), Firmicutes (15%), Planctomycetes (2%), and Cyanobacteria (0.3%). Among these, Proteobacteria exhibited the highest bacterial abundance among the analyzed samples (Figure 1).

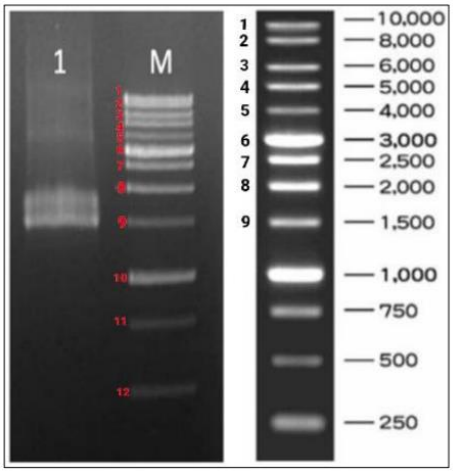

Figure 1 Gel Electrophoresis Results of Bacterial DNA Amplification of Passive Cell Soil Samples at Bestari Landfill, Probolinggo City

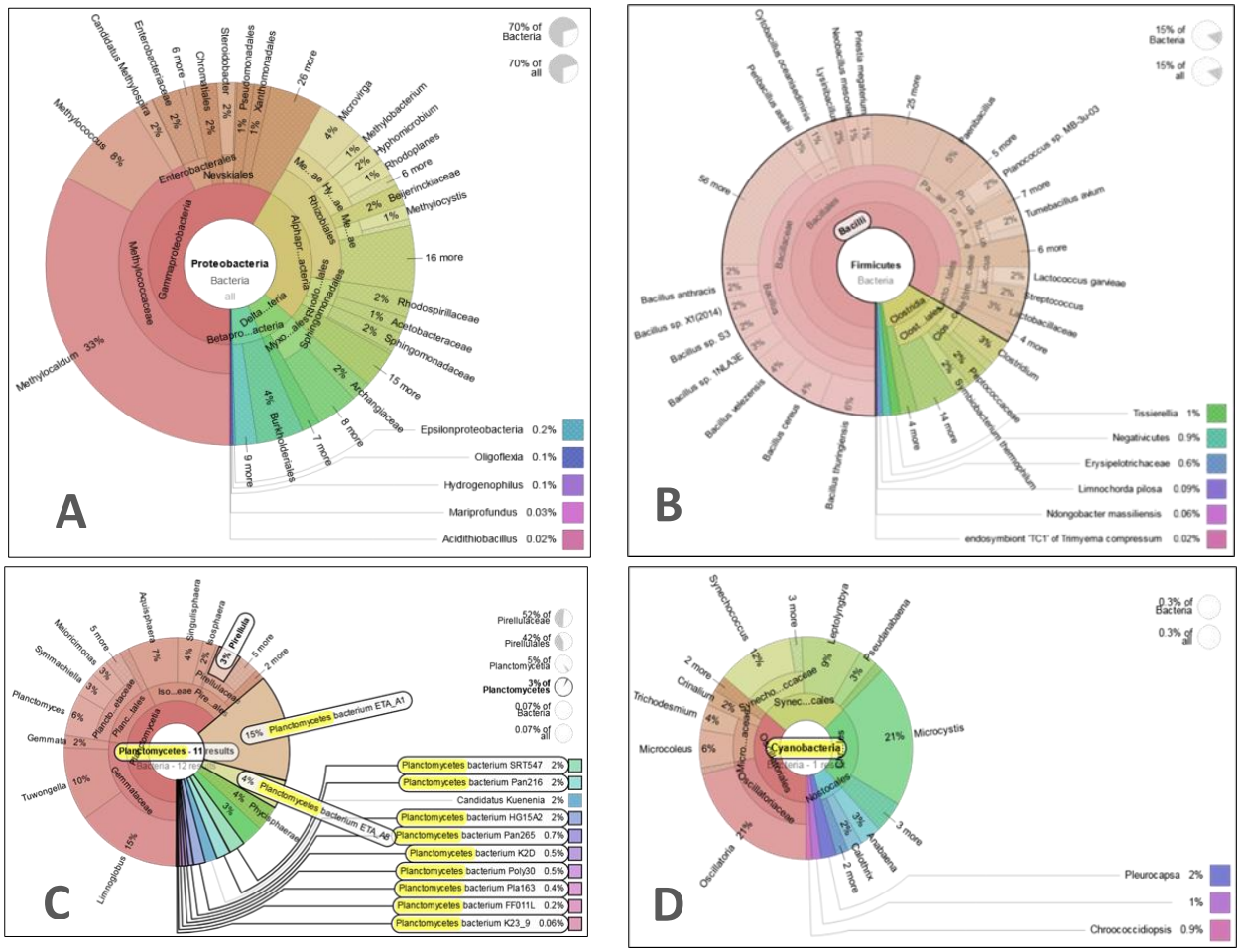

Figure 2. Distribution of (A) Proteobacteria (70%), (B) Firmicutes (15%), (C) Planctomycetes (2%), and (D) Cyanobacteria (0.3%) phylum

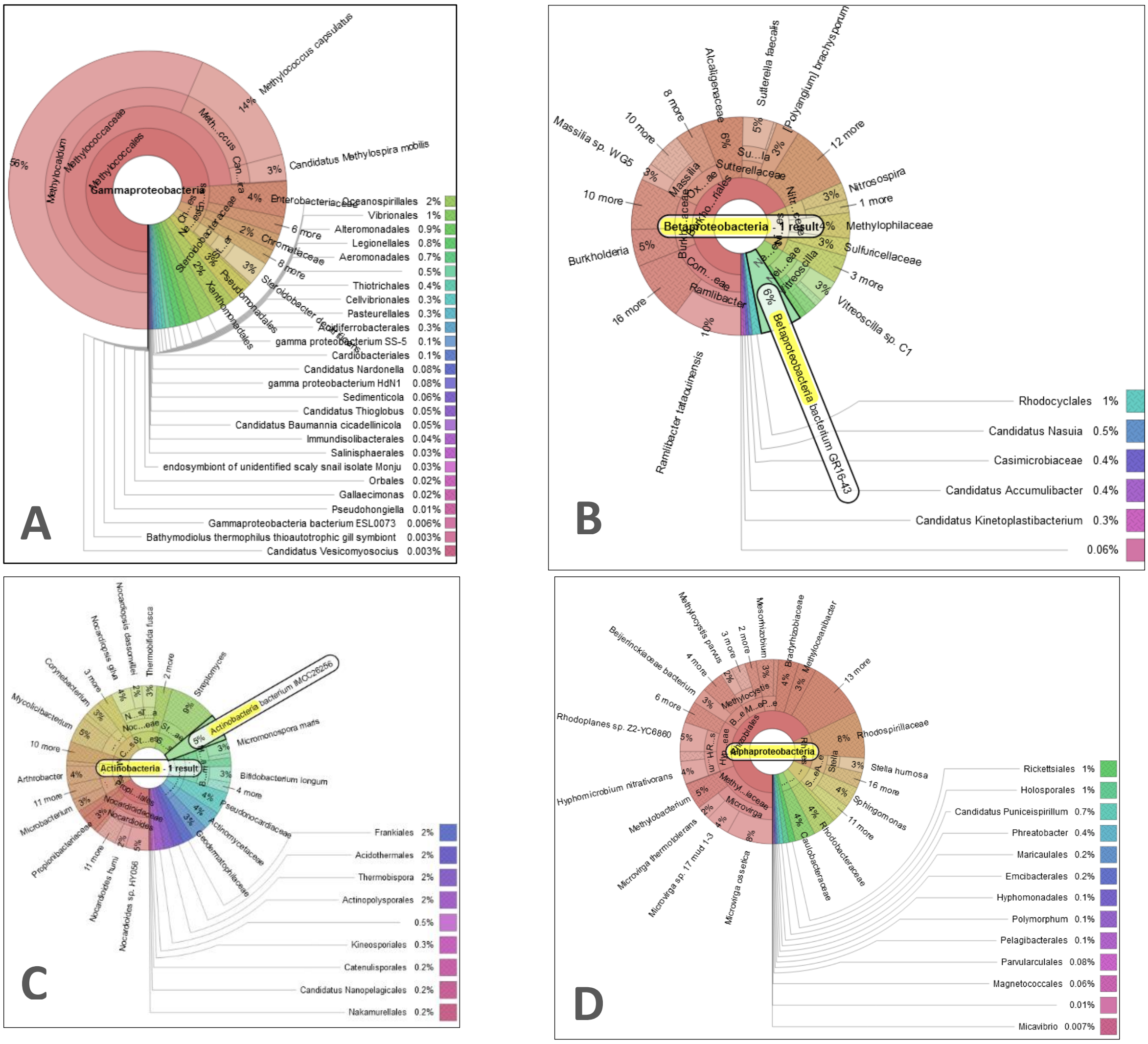

Figure 3. Class distribution of (A) Gammaproteobacteria, (B) Betaproteobacteria, (C) Actinobacteria, and (D) Alphaproteobacteria

Proteobacteria are a diverse group of bacteria that can exhibit various metabolic characteristics, including photosynthetic, heterotrophic, or chemolithotrophic modes of nutrition. These bacteria are typically Gram-negative and may possess flagella or lack flagella. Their cell shapes can vary and include rods, spiral, or round forms. Proteobacteria can thrive in aerobic, facultative anaerobic, or anaerobic conditions [22]. According to Gao et al. (2016), Proteobacteria, such as Pseudomonas, are commonly found in environments with a pH range of 5.5 to 8.2. Optimal growth temperatures for Proteobacteria, including Pseudomonas, typically fall within the range of 20 to 37˚C [23]. The environmental conditions in which the soil samples were collected, with a pH range of approximately 6.9 to 7 and temperatures ranging from 30 to 36˚C, are conducive to the growth and development of Proteobacteria. The NGS analysis results revealed the presence of four classes in the passive cell soil samples of the Bestari landfill. These classes are Gammaproteobacteria, Betaproteobacteria, Actinobacteria, and Alphaproteobacteria, as depicted in Figure 2.

The diversity of orders based on the results of the NGS analysis was obtained in as many as 17 ordos, including Enterobacteriaceae, Chitinophagaceae, Bacillaceae, and Comamonadaceae, Acidobacteriaceae, Sphingobacteriaceae, Oxalobacteraceae, Rhodospirillaceae, Rhodobacteraceae, Microbacteriaceae, Alcaligenaceae, Lachnospiraceae, Sphingomonadaceae, and Propionaceae, Acetobacteriaceae. Also as many as 56 genera (Figure 3).

Exploration The Candidates of Xenobiotic Degrading Indigenous Bacteria from Probolinggo City Landfill by Using Next Generation Sequencing (NGS)

Xenobiotic compounds are foreign substances that enter or are introduced into living

things. In health, xenobiotics are foreign substances entering the human body. In contrast, in the environment, xenobiotics are known as pollutants that enter the environment, including soil, water, and gas. These compounds have many types, including drugs, insecticides, and various cancer-triggering compounds. The xenobiotic compounds that are currently becoming a crucial problem i.e. polypropylene, ammonia, styrofoam, profenofos, chlorantraniliprole and tannic acid. Several species that have been known to have the potential to degrade xenobiotic compounds are identified in the NGS results as conveyed in the following description.

Jain et al. (2018), showed that their study identified five isolates from compost in the Okhla landfill in New Delhi that exhibited potential for degrading polypropylene. These isolates were identified as *Bacillus cereus, B. licheniformis,* and *B. thuringiensis*. Among these isolates also promising evidence for the biodegradation of polypropylene and poly-L-lactide (PP-PLLA) mixtures in the environment by Bacillus species isolated from composted samples. Based on NGS analysis, only the species *Bacillus cereus*, and *B. thuringiensis* were identified in the passive cell area of Bestari Probolinggo City landfill.

Hidayat et al. (2020) successfully isolated bacteria capable of degrading styrofoam. The study observed bacterial biofilms growing on the surface of styrofoam and identified four species of styrofoam-degrading bacteria, namely *B. amyloliquefaciens, B. cereus, B. firmus*, and *P. aeruginosa*. Based on a NGS analysis, only the species *B. amyloliquefaciens, B. cereus, and P. aeruginosa* were identified in the passive cell area of Bestari Probolinggo City landfill.

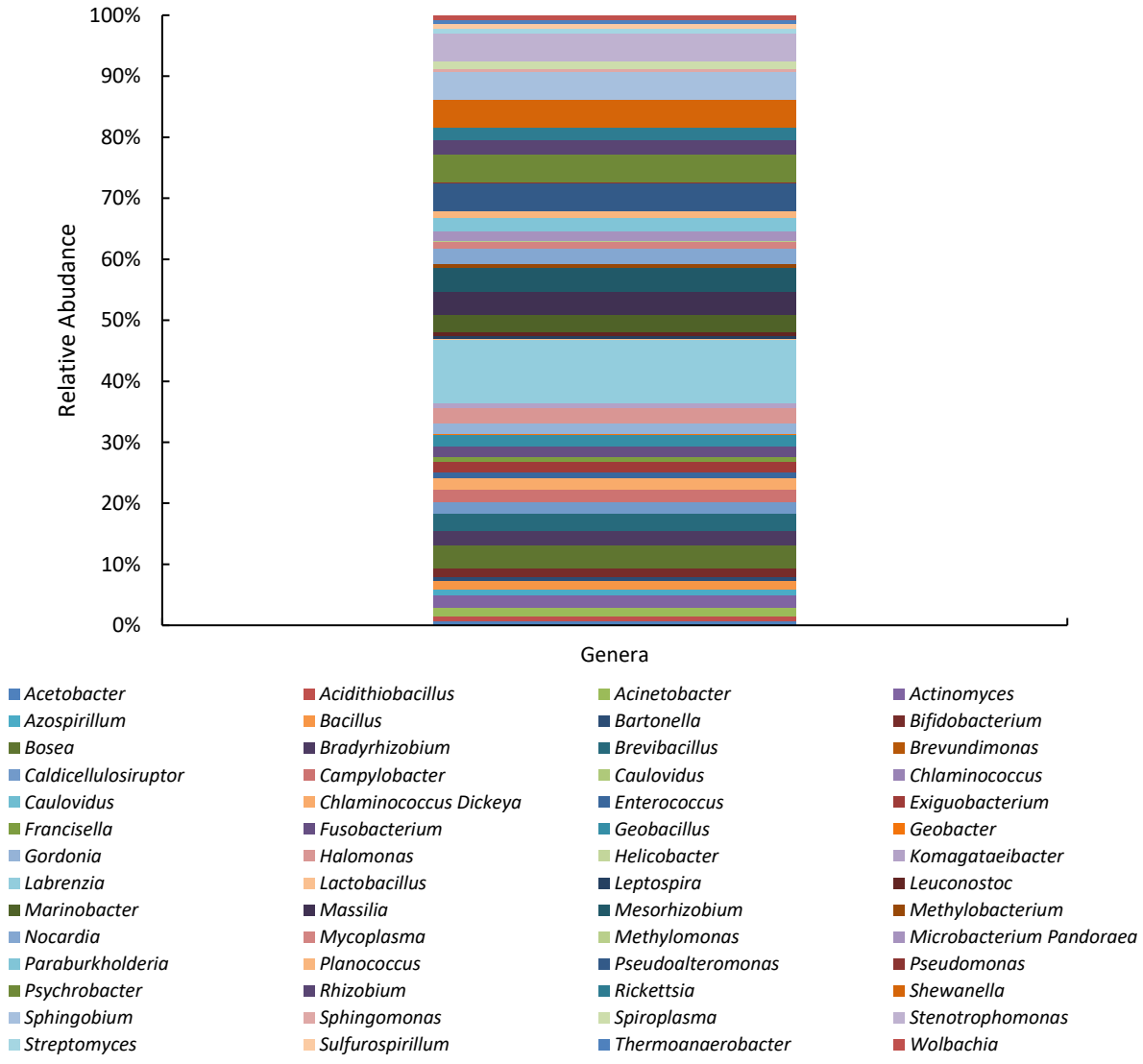

Figure 4. Biodiversity 56 genera in the passive cell area Probolinggo City landfill

Yuka et al. (2021) shows that *Bacillus megaterium* from the sediment samples taken from a shrimp pond in Pasir Sakti, East Lampung potential for bioremediation applications, particularly in reducing TAN in shrimp pond waste. This species also identified in the of Bestari Probolinggo City landfill based on NGS analysis.

Pratiwi et al. (2022) found three bacterial isolates that were isolated and identified as *Bacillus stearothermophilus, Bacillus badius,* and *Bacillus alvei*, with the results of the percentage accuracy of similarity coefficients of 70%, 70%, and 60%. The three isolates of indigenous bacteria that have been identified can be used as bioremediation agents on agricultural soil contaminated with the pesticides profenofos and chlorantraniliprole [24]. Based on NGS analysis, only the species *Bacillus stearothermophilus* were identified in the of Bestari Probolinggo City landfill.

Devi, M. (2021) obtained 49 isolates of tannic acid-degrading microbes. The K12 isolate was the selected isolate, which had the highest tannic acid degradation activity and cell growth, as well as less tannic acid residue in the medium than other isolates. Morphological, biochemical identification, and analysis of 16S rRNA gene sequences showed that isolated K12 was a bacterium *Pantoea dispersa* [25]. Based on NGS analysis, only the bacterium *Pantoea dispersa* was identified in the passive cell area of Bestari Probolinggo City landfill.

Interestingly, based on a comparative narrative review analysis with NGS analysis shows that the diversity of bacterial species in the Bestari landfill has various potential benefits bacteria as sources of degrading bacteria polypropylene, and styrofoam, reduce Total Ammonia Nitrogen (TAN), The pesticide profenofos and chlorantraniliprole, also tannic acid.

4. CONCLUSIONS

The results show that the soil sample of the Bestari landfill, Probolinggo City has a very diverse body of bacteria. Based on Next Generation Sequencing (NGS) analysis, there were 2400 bacterial species consisting of 56 genera, 17 orders, four classes, and four phyla with their respective abundances: Proteobacteria (70%), Firmicutes (15%), Planctomycetes (2 %), and Cyanobacteria (0.3%). Based on a comparative narrative review analysis with NGS analysis shows that the diversity of bacterial species in the Probolinggo City landfill has various potential benefits bacteria as sources of degrading bacteria polypropylene (*Bacillus cereus*, *B. licheniformis*, and *B. thuringiensis*), and styrofoam (species *B. amyloliquefaciens, B. cereus, and P. aeruginosa*), reduce TAN (*Bacillus megaterium*), the pesticide profenofos and chlorantraniliprole (*Bacillus stearothermophilus*), also tannic acid (*Pantoea dispersa*).

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